GENETICS

Supporting Information
http://www.genetics.org/cgi/content/full/genetics.110.119628/DC1

Drosophila Mis12 Complex Acts as a Single Functional Unit Essential for Anaphase Chromosome Movement and a Robust Spindle Assembly Checkpoint

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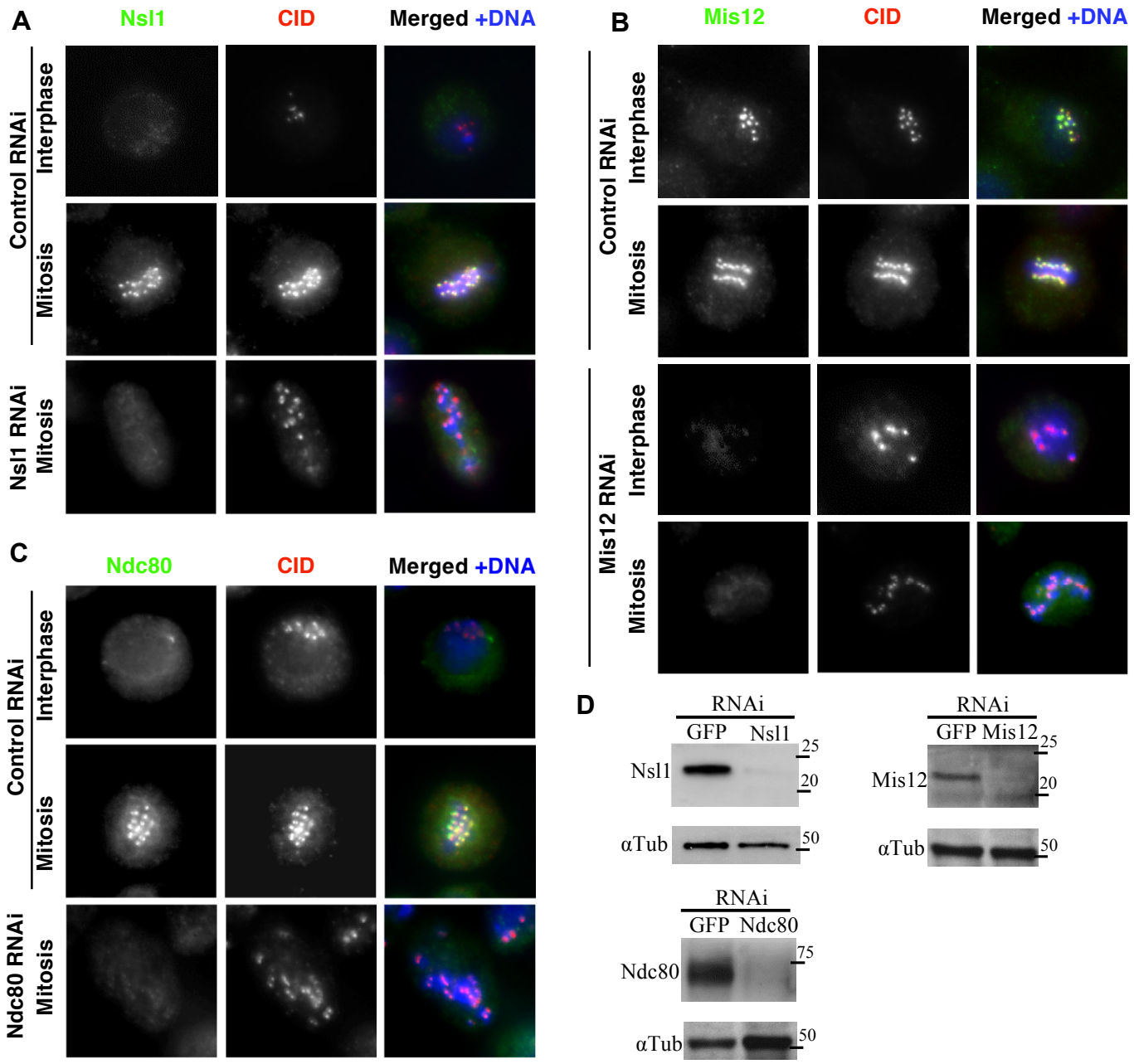


FIGURE S1.—Immunolocalisation (A-C) and Western blotting results (D) of Nsl1, Mis12 or Ndc80 in Dmel cells. Nsl1 (A), Mis12 (B), or Ndc80 (C) are in green; CID, red; and DNA, blue. Immunostaining is lost following depletion of the appropriate protein by RNAi. (D) Specific bands on Western blots disappear after Nsl1, Mis12 or Ndc80 RNAi.

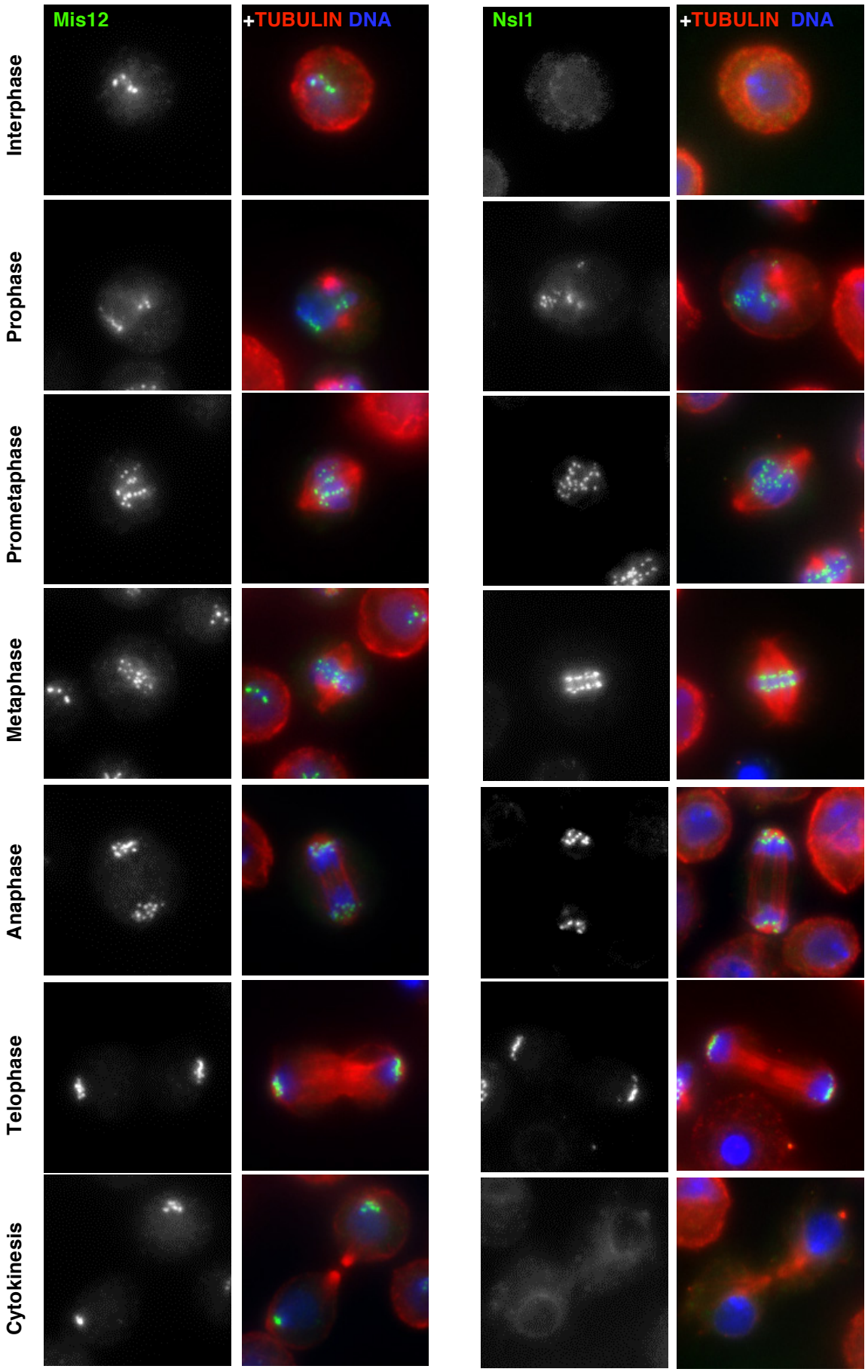


FIGURE S2.—Localization of endogenous Mis12 and Nsl1 proteins in the Dmel cell cycle. Dmel cells are stained to reveal Mis12 or Nsl1 (green), tubulin (red) and DNA (blue).

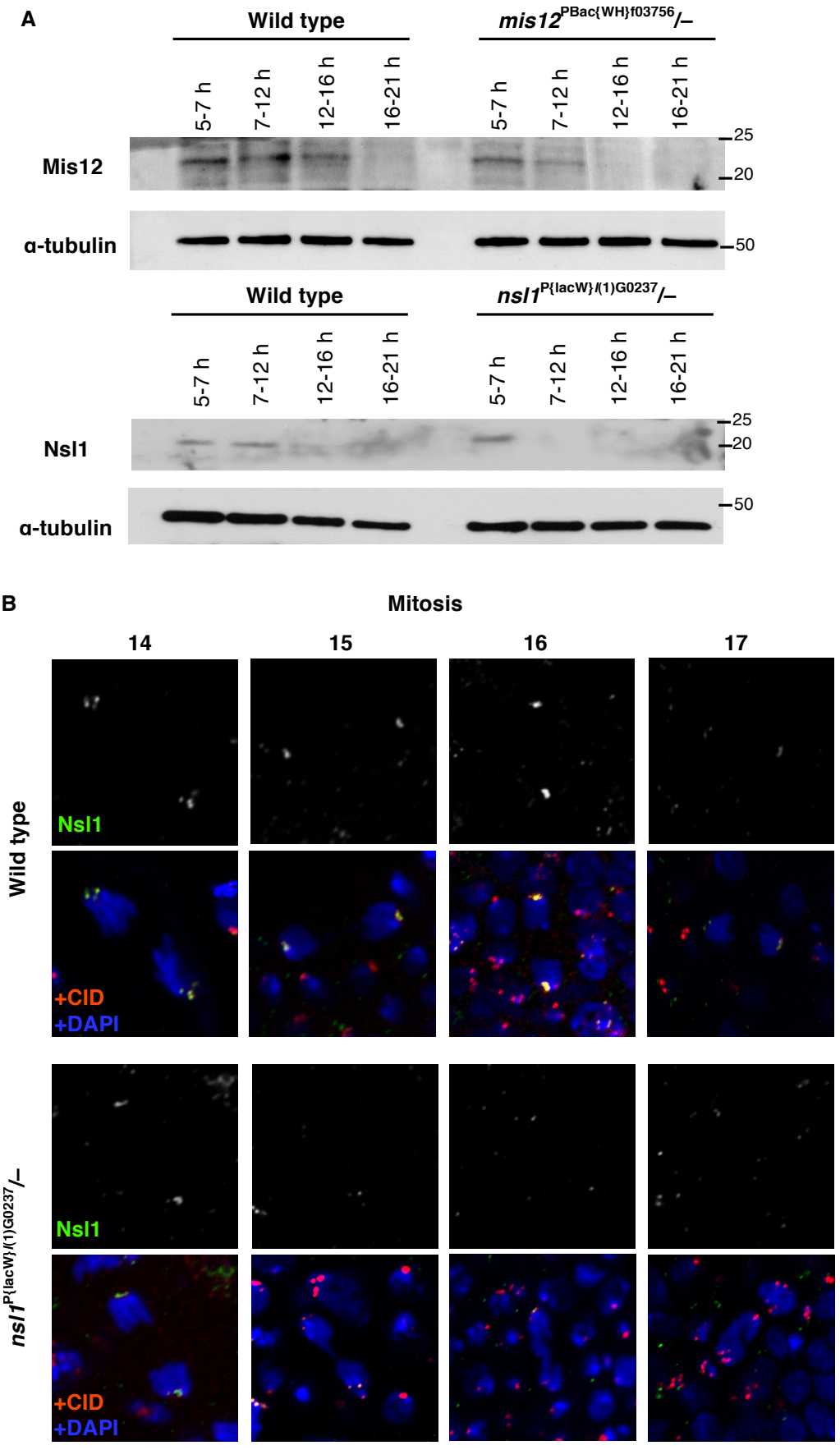
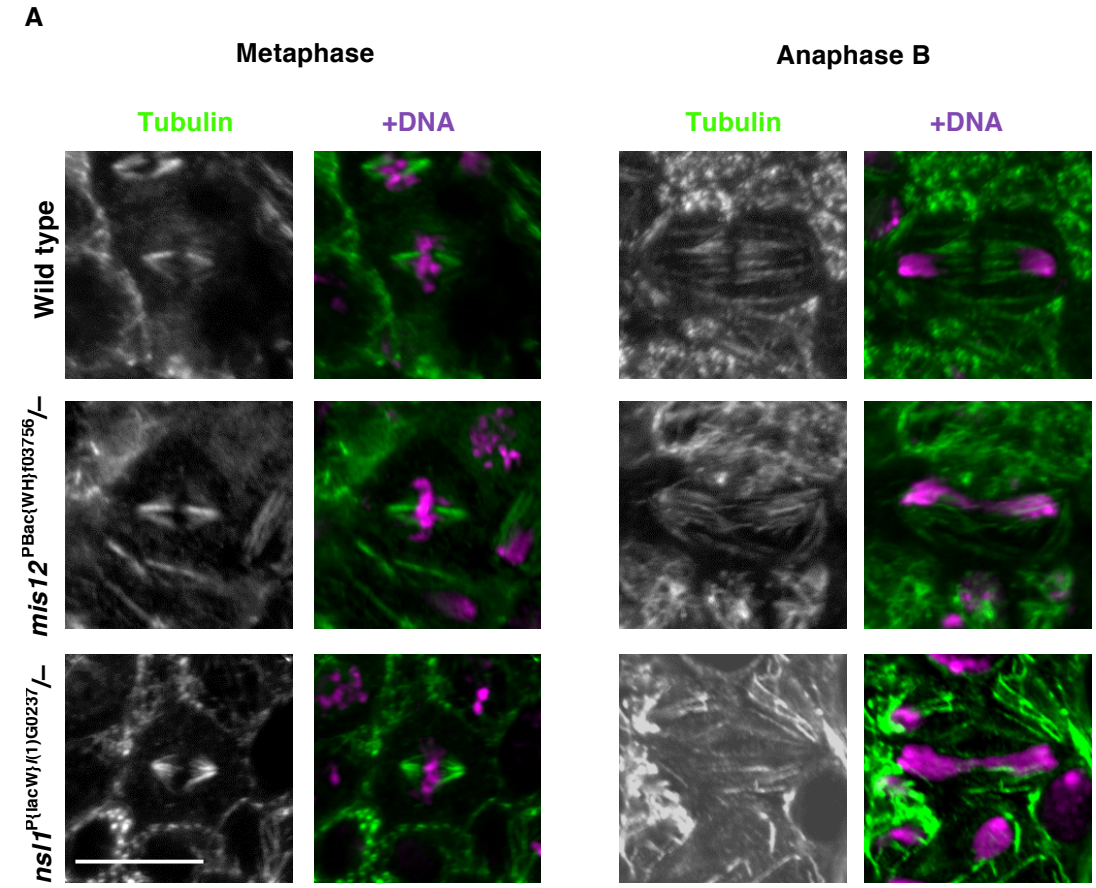


FIGURE S3.—Progressive loss of Nsl1 and Mis12 in mutant embryos— (A) Western blot to compare Mis12 and Nsl1 protein levels in wild type and *mis12 or nsl1* mutant embryos between 5 – 21h. (B) Immuno-localization of Nsl1 (green) in successive cycles of cellularised wild type and *nsl1* mutant embryos. Embryos are counterstainined to reveal CID (red) and DNA (blue).


A
Metaphase
Anaphase B
Tubulin
+DNA
Tubulin
+DNA
Wild type
mis12PBac{WH}f03756/–
nsl1P{lacW}l(1)G0237/–

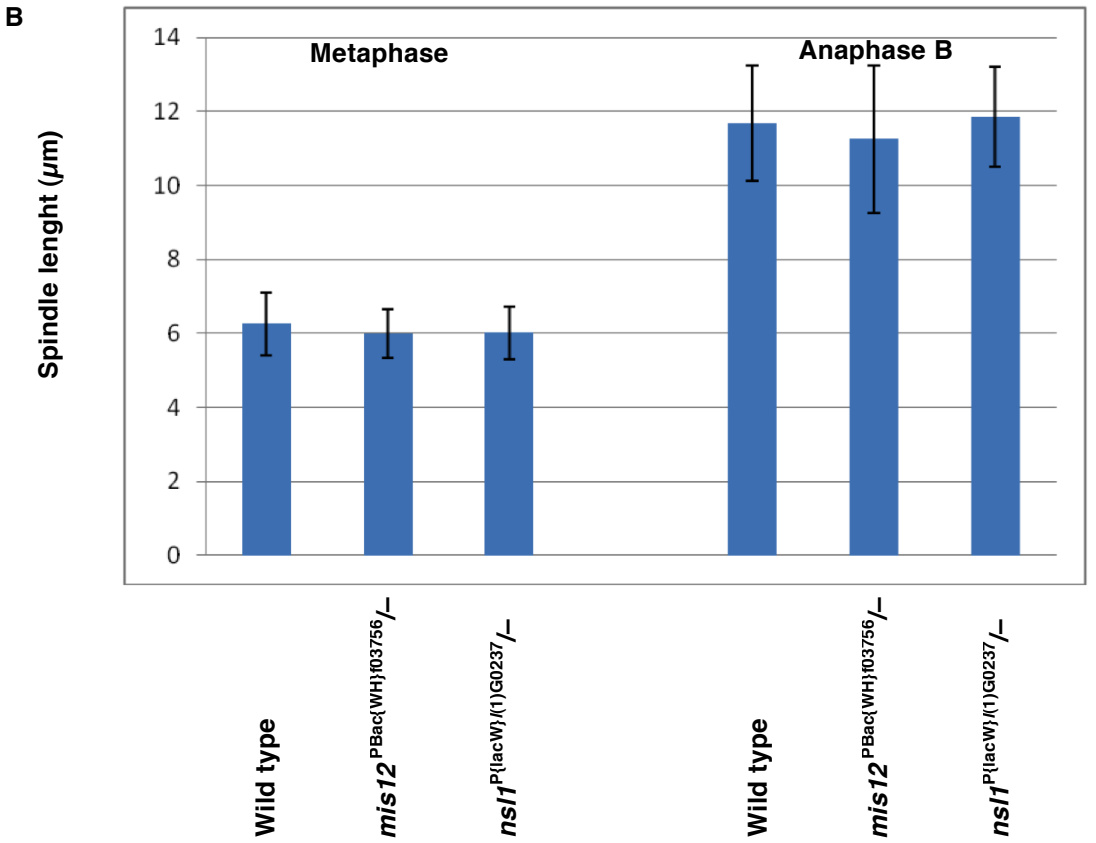

B
Spindle lenght (μm)
14
12
10
8
6
4
2
0
Metaphase
Anaphase B
Wild type
mis12PBac{WH}f03756/–
nsl1P{lacW}l(1)G0237/–
Wild type
mis12PBac{WH}f03756/–
nsl1P{lacW}l(1)G0237/–

FIGURE S4.—Organisation and length of mitotic spindles in *nsl1* and *mis12* mutant embryos— (A) Mitotic spindles in *mis12* and *nsl1* mutant embryos in mitosis 15. Scale bar 10 μm. (B) Length of spindles in metaphase and anaphase B of mitosis 15. Error bars represent ±1 SD. There is no statistically significant difference in length between wild type and mutant spindles either in meta- or in anaphase B (two-tailed independent t-test, α=0.05, n=15 for all genotype and mitotic phase).

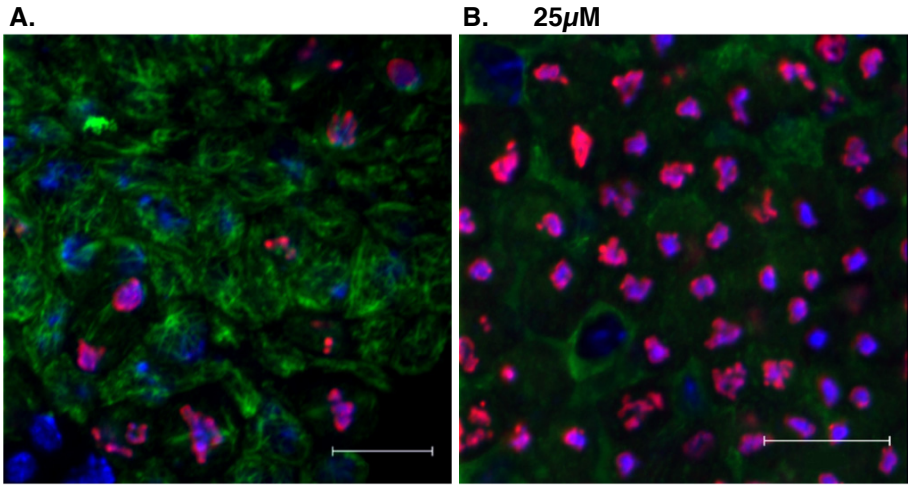


FIGURE S5.—Checkpoint response of wild-type embryos to colcemid treatment. Mitotic cells in stage 14 wild-type embryos treated for 1h with DMSO (A) or 25μM Colcemid (B) before fixation. Staining to reveal Ser-10-PH3 is in red; α-tubulin, green; and DNA, blue. Scale bars represent 10μm.

TABLE S1

Features of *UASp-Mis12::EGFP* and *UASp-Nsl1::EGFP* transgenes

Transgene	Tested lines	Homozygous	Driven by *actin5C-Gal4(2)*[a]
UASp-Mis12::EGFP	2 lines with single copies of the transgene on the 2nd chromosome	Viable, fertile	Renders viability of *mis12*$^{PBac\{WH\}f03756}$*/Df(3L)BSC224* flies
UASp-Nsl1::EGFP	3 lines with single copies of the transgene on the 3rd chromosome	Viable, fertile	Renders viability of *nsl1*$^{P\{lacW\}G0237}$*/–* flies

[a] (ITO *et al.* 1997)

FILES S1-S11

Supporting Movies

Files S1-S11 are available for download as .avi files at http://www.genetics.org/cgi/content/full/genetics.110.119628/DC1. In each movie EGFP tagged proteins are shown in cyan pseudo colour and tubulin in red. Frames of files S1-6 were taken in every 18 seconds and frames of files 9-13 in every 20 seconds. Movies play with 10 frames/second.

File S1: Mis12-EGFP localization throughout nuclear cleavage division 12.

File S2: Mis12-EGFP localisation after cellularization in mitosis 14 and 15.

File S3: Nsl1-EGFP localization throughout nuclear cleavage division 12.

File S4: Nsl1-EGFP localization in mitosis 14 and 15 in cellularized embryo.

File S5: Nuf2-EGFP localization throughout nuclear cleavage division 12.

File S6: Nuf2-EGFP localization in mitosis 14 and 15 in cellularized embryo.

File S7: *His2Av-EGFP (2)* embryos in Mitosis 15.

File S8: *His2Av-EGFP* (2); *Mis12*$^{PBac\{WH\}f03756}$*/– (3)* embryo in mitosis 15 with weak chromosome segregation defects.

File S9: *His2Av-EGFP* (2); *Mis12*$^{PBac\{WH\}f03756}$*/– (3)* embryo in mitosis 15 with strong chromosome segregation defects (cut phenotype).

File S10: *His2Av-EGFP* (2); *Mis12*$^{PBac\{WH\}f03756}$*/– (3)* embryo in mitosis 15 with metaphase arrested cells.

File S11: *Nsl1*$^{P\{lacW\}G0237}$*/–* ; *His2Av-EGFP (2)* embryo in mitosis 15 with chromosome segregation defects.

Supporting reference

ITO, K., W. AWANO, K. SUZUKI, Y. HIROMI and D. YAMAMOTO, 1997 The Drosophila mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells. Development **124:** 761-771.